

Anti-Human CD44 (HP2/9)



PURE	44PUI	1 mg	1 mg/ml
FITC	44FI-100T	100 test	20 µL/test 2 mg/ml
PE	44PEI-100T	100 test	20 µL/test 2 mg/ml

RUO

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

Please, refer to www.immunostep.com technical support for more information.

1. PRODUCT DESCRIPTION

Clone: HP2/9;
Isotype: IgG1;
Tested application: flow cytometry;
Immunogen: T cell leukemia HPB-ALL;
Species reactivity: Human;
Storage instruction: store in the dark at 2-8 °C;
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN₃);
Recommended usage: Immunostep's CD44, clone HP2/9 is a monoclonal antibody intended for the identification and enumeration of leucocytes and erythrocytes using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 10⁶ cells;
Presentation: liquid;
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;
Specificity: The antibody specifically recognizes the human protein CD44 and it was validated by the 5th International Workshop on Human Leucocyte Differentiation Antigens (Boston, USA, 3-7 November 1993). Clone HP2/9 is capable of blocking the binding of hyaluronic acid to cells^(1,4) in its standard isoform (CD44s), probably binding to more than one site (5) on CD44.
 Clone HP2/9 is capable of blocking clone LI78 but not clone HI44a, thereby recognizing different epitopes than clone HI44a. LI78 clone does not block HP2/9 clone.
Purification: Affinity chromatography;
Other names: CDw44, Epcan, Extracellular matrix receptor III, ECMR-III, GP90 lymphocyte homing/adhesion receptor, HUTCH-I, Heparan sulfate proteoglycan, Hermes antigen, Hyaluronate receptor, Phagocytic glycoprotein I, PGP-I, Phagocytic glycoprotein I, Pgp-I, H-CAM, gp85, Ly-24;
Gene ID: 960;
Molecular weight: 80 - 95 kDa.

2. ANTIGEN DETAILS

Large description: The monoclonal antibody is directed against the CD44- antigen, which is mainly expressed on leucocytes and erythrocytes. Very few types of tissue or cells lack CD44. Cells and tissues that lack CD44 include platelets, hepatocytes, certain lymphoid cell lines, cardiac muscle, kidney tubular epithelium, testis and portions of the skin. The CD44 antigen is present on approximately 90% of lymphocytes, monocytes, and granulocytes and in lower amounts on thymocytes, fibroblasts, and erythrocytes.

The antigen mediates a variety of functions, including leucocyte-endothelial cell binding and lymphocyte homing to certain peripheral lymphoid microenvironments.⁽¹⁻³⁾

3. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties.

Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

4. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

5. PROTOCOL

■ Direct Immunofluorescence Cell Surface Staining Protocol

1. Transfer 100 µl (10⁶ cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
3. Mix well and incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution).
5. Centrifuge tubes at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
6. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
7. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and acquire on the flow cytometer are recorded.
8. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

■ Indirect Immunofluorescence Cell Surface Staining Protocol

1. Transfer 100 µl (10⁶ cells/test) of the sample to a 12 x 75 mm polystyrene test tube
2. Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
3. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
5. Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for 15 min in the dark. The absence of light is necessary as the fluorochrome is photoinstability.
6. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution). Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
7. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
8. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and acquire on the flow cytometer are recorded.
9. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

6. REFERENCES

1. Telen MJ, Udani M, Washington MK, Levesque MC, Lloyd E, Rao N. A blood group-related polymorphism of CD44 abolishes a hyaluronan-binding consensus sequence without preventing hyaluronan binding. *J Biol Chem.* 1996 Mar 22;271(12):7147-53. doi: 10.1074/jbc.271.12.7147. PMID: 8636151.
2. de la Hera A, Acevedo A, Marston W, Sanchez-Madrid F. Function of CD44(Pgp-I) homing receptor in human T cell precursors. *Int Immunol.* 1989;1(6):598-604. doi: 10.1093/intimm/1.6.598. PMID: 2489047.








3. Lesley J, Hyman R, Kincade PW. CD44 and its interaction with extracellular matrix. *Adv Immunol*1993;54:271-335.

4. García-Peydró M, Fuentes P, Mosquera M, García-León MJ, Alcain J, Rodríguez A, García de Miguel P, Menéndez P, Weijer K, Spits H, Scadden DT, Cuesta-Mateos C, Muñoz-Calleja C, Sánchez-Madrid F, Toribio ML. The NOTCH1/CD44 axis drives pathogenesis in a T cell acute lymphoblastic leukemia model. *J Clin Invest.* 2018 Jul 2;128(7):2802-2818. doi: 10.1172/JCI92981. Epub 2018 May 21. PMID: 29781813; PMCID: PMC6025994.

5. Liao HX, Lee DM, Levesque MC, Haynes BF. N-terminal and central regions of the human CD44 extracellular domain participate in cell surface hyaluronan binding. *J Immunol.* 1995 Oct 15;155(8):3938-45. PMID: 7561101.

6. Ugarte-Berzal E, Bailón E, Amigo-Jiménez I, Albar JP, García-Marco JA, García-Pardo A. A novel CD44-binding peptide from the pro-matrix metalloproteinase-9 hemopexin domain impairs adhesion and migration of chronic lymphocytic leukemia (CLL) cells. *J Biol Chem.* 2014 May 30;289(22):15340-9. doi: 10.1074/jbc.M114.559187. Epub 2014 Apr. PMID: 24739387; PMCID: PMC4140891.

7. EXPLANATION OF SYMBOLS

	Form
	Catalog reference
	Contains sufficient for <n> test
	Quantity per test
	Regulatory Status
	Research Use Only
	Manufacturer

8. MANUFACTURED BY:



Address: Avda. Universidad de Coimbra, s/n
 Cancer Research Center (C.I.C)
 Campus de Unamuno
 37007 Salamanca (Spain)
Tel./fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com